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(54) Title: METHOD OF DIAGNOSING, MONITORING, STAGING, IMAGING AND TREATING GYNECOLOGIC CANCERS AND TESTICULAR CANCER (57) Abstract The present invention provides a new method for detecting, diagnosing, monitoring, staging, prognosticating, imaging and treating gynecologic cancers and testicular cancer.		

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**METHOD OF DIAGNOSING,
MONITORING, STAGING, IMAGING AND TREATING GYNECOLOGIC
CANCERS AND TESTICULAR CANCER**

FIELD OF THE INVENTION

5 This invention relates, in part, to newly developed assays for detecting, diagnosing, monitoring, staging, prognosticating, imaging and treating cancers, particularly gynecologic cancers including ovarian, mammary, endometrial, uterine and cervical cancer, and testicular cancer.

10 **BACKGROUND OF THE INVENTION**

 In women, gynecologic cancers account for more than one-fourth of the malignancies.

 Of the gynecologic cancers, breast cancer is the most common. According to the Women's Cancer Network, 1 out of
15 every 8 women in the United States is at risk of developing breast cancer, and 1 out of every 28 women are at risk of dying from breast cancer. Approximately 77% of women diagnosed with breast cancer are over the age of 50. However, breast cancer is the leading cause of death in women
20 between the ages of 40 and 55.

 Carcinoma of the ovary is another very common gynecologic cancer. Approximately one in 70 women will develop ovarian cancer during her lifetime. An estimated 14,500 deaths in 1995 resulted from ovarian cancer. It causes more deaths than
25 any other cancer of the female reproductive system. Ovarian cancer often does not cause any noticeable symptoms. Some possible warning signals, however, are an enlarged abdomen due to an accumulation of fluid or vague digestive disturbances (discomfort, gas or distention) in women over 40; rarely there
30 will be abnormal vaginal bleeding. Periodic, complete pelvic examinations are important; a Pap test does not detect ovarian

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cancer. Annual pelvic exams are recommended for women over 40.

Also common in women is endometrial cancer or carcinoma of the lining of the uterus. According to the Women's Cancer Center endometrial cancer accounts for approximately 13% of all malignancies in women. There are about 34,000 cases of endometrial cancer diagnosed in the United States each year.

Uterine sarcoma is another type of uterine malignancy much more rare as compared to other gynecologic cancers. In uterine sarcoma, malignant cells start growing in the muscles or other supporting tissues of the uterus. Sarcoma of the uterus is different from cancer of the endometrium, a disease in which cancer cells start growing in the lining of the uterus. This uterine cancer usually begins after menopause. Women who have received therapy with high-dose X-rays (external beam radiation therapy) to their pelvis are at a higher risk to develop sarcoma of the uterus. These X-rays are sometimes given to women to stop bleeding from the uterus.

Cancer of the cervix, another common kind of cancer in women, is a disease in which cancer (malignant) cells are found in the tissues of the cervix. Cancer of the cervix usually grows slowly over a period of time. Before cancer cells are found on the cervix, the tissues of the cervix undergo changes in which abnormal cells begin to appear (known as dysplasia). A Pap smear will usually identify these cells. Later, cancer cells begin to grow and spread more deeply into the cervix and to surrounding areas. Since there are usually no symptoms associated with cancer of the cervix, accurate diagnostic tests are critical. The first of these is a Pap smear, which is done by using a piece of cotton, a brush, or a small wooden stick to gently scrape the outside of the cervix in order to pick up cells. If cells that are not normal are found, the doctor will remove a sample of tissue (this procedure is called a biopsy) from the cervix and examine it microscopically for cancer cells. A patient may

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need to go to the hospital if a larger, cone-shaped biopsy specimen (conization) is required. The prognosis (chance of recovery) and choice of treatment depend on the stage of the cancer (whether it is just in the cervix or has spread to other places) and the patient's general health.

The lifetime probability of developing testicular cancer is 0.2% for an American white male. The cause of testicular cancer is unknown, however, it is associated with both congenital and acquired factors. From a treatment standpoint, testicular cancer is divided into two major categories, nonseminomas and seminomas. In the commonly used staging system for nonseminomas, a stage A lesion is confined to the testis; in stage B there is regional lymph node involvement in the retroperitoneum; and in stage C, there is distant metastasis. For seminomas, a stage I lesion is confined to the testis; in stage II, the lesion has spread to the retroperitoneal lymph nodes; and in stage III, the lesion has supradiaphragmatic nodal or visceral involvement.

The most common symptom of testicular cancer is painless enlargement of the testis. Acute testicular pain resulting from intertesticular hemorrhage occurs in about 10% of cases. Patients are often asymptomatic upon presentation but about 10% may exhibit back pain, cough or lower extremity edema. A testicular mass or diffuse enlargement of the testis can be detected by physical examination in most cases. Several biochemical markers are used for the diagnosis and treatment of this disease including human chorionic gonadotropin (hCG), alpha-fetoprotein and LDH. However, an incorrect diagnosis is made initially in up to 25% of patients with testicular tumors. Accordingly, improved methods for diagnosing testicular cancer are needed.

Procedures used for detecting, diagnosing, monitoring, staging, and prognosticating these cancers are of critical importance to the outcome of the patient. Patients diagnosed early generally have a much greater five-year survival rate

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as compared to the survival rate for patients diagnosed with distant metastasized cancer. New diagnostic methods which are more sensitive and specific for detecting early cancers are clearly needed.

5 Cancer patients are closely monitored following initial therapy and during adjuvant therapy to determine response to therapy and to detect persistent or recurrent disease or metastasis. Thus, there is also clearly a need for cancer markers which are more sensitive and specific in detecting
10 cancer recurrence.

Another important step in managing cancer is to determine the stage of the patient's disease. Stage determination has potential prognostic value and provides criteria for designing optimal therapy. Generally, pathological staging of cancer
15 is preferable over clinical staging because the former gives a more accurate prognosis. However, clinical staging would be preferred were it at least as accurate as pathological staging because it does not depend on an invasive procedure to obtain tissue for pathological evaluation. Staging of
20 cancer would be improved by detecting new markers in cells, tissues or bodily fluids which could differentiate between different stages of invasion.

In the present invention, methods are provided for detecting, diagnosing, monitoring, staging, prognosticating,
25 imaging and treating gynecologic cancers or testicular cancer via a Cancer Specific Gene (CSG). The CSG refers, among other things, to native protein expressed by the gene comprising the polynucleotide sequence of SEQ ID NO:1. The amino acid sequence of a polypeptide encoded by SEQ ID NO:1 is depicted
30 herein as SEQ ID NO:2. In the alternative, what is meant by the CSG as used herein, means the native mRNA encoded by the gene comprising the polynucleotide sequence of SEQ ID NO:1 or levels of the gene comprising the polynucleotide sequence of SEQ ID NO:1.

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Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the
5 specific examples, while indicating preferred embodiments of the invention are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and
10 from reading the other parts of the present disclosure.

SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide a method for diagnosing the presence of gynecologic cancers or testicular cancer by
15 analyzing for changes in levels of CSG in cells, tissues or bodily fluids compared with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein a change in levels of CSG in the patient versus the normal human control is associated with a
20 gynecologic cancer or testicular cancer.

Further provided is a method of diagnosing metastatic cancer in a patient having a gynecologic cancer or testicular which is not known to have metastasized by identifying a human patient suspected of having a gynecologic cancer or testicular
25 cancer that has metastasized; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein an increase in
30 CSG levels in the patient versus the normal human control is associated with a gynecologic cancer or a testicular cancer which has metastasized.

Also provided by the invention is a method of staging gynecologic cancers or testicular cancer in a human which has

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such cancer by identifying a human patient having such cancer; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Further provided is a method of monitoring gynecologic cancers or testicular cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

Further provided is a method of monitoring the change in stage of a gynecologic cancer or testicular cancer in a human having such cancer by looking at levels of CSG in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

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Further provided are antibodies against CSG or fragments of such antibodies which can be used to detect or image localization of CSG in a patient for the purpose of detecting or diagnosing a disease or condition. Such antibodies can be polyclonal, monoclonal, or omniclonal or prepared by molecular biology techniques. The term "antibody", as used herein and throughout the instant specification is also meant to include aptamers and single-stranded oligonucleotides such as those derived from an *in vitro* evolution protocol referred to as SELEX and well known to those skilled in the art. Antibodies can be labeled with a variety of detectable labels including, but not limited to, radioisotopes and paramagnetic metals. These antibodies or fragments thereof can also be used as therapeutic agents in the treatment of diseases characterized by expression of a CSG. In therapeutic applications, the antibody can be used without or with derivatization to a cytotoxic agent such as a radioisotope, enzyme, toxin, drug or a prodrug.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to diagnostic assays and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging and prognosticating cancers by comparing levels of CSG with those of CSG in a normal human control. What is meant by levels of CSG as used herein, means

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levels of the native protein expressed by the gene comprising the polynucleotide sequence of SEQ ID NO:1. The amino acid sequence of a polypeptide encoded by SEQ ID NO:1 is depicted herein as SEQ ID NO:2. In the alternative, what is meant by
5 levels of CSG as used herein, means levels of the native mRNA encoded by the gene comprising the polynucleotide sequence of SEQ ID NO:1 or levels of the gene comprising the polynucleotide sequence of SEQ ID NO:1. Such levels are preferably measured in at least one of, cells, tissues and/or
10 bodily fluids, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for diagnosing over-expression of CSG protein compared to normal control bodily fluids, cells, or tissue samples may be used to diagnose the presence of
15 cancers, including gynecologic cancers such as breast, ovarian, uterine, endometrial and cervical cancer, and testicular cancer.

All the methods of the present invention may optionally include measuring the levels of other cancer markers as well
20 as CSG. Other cancer markers, in addition to CSG, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art.

Diagnostic Assays

The present invention provides methods for diagnosing the
25 presence of gynecologic cancers or testicular cancer by analyzing for changes in levels of CSG in cells, tissues or bodily fluids compared with levels of CSG in cells, tissues or bodily fluids of preferably the same type from a normal human control, wherein an increase in levels of CSG in the
30 patient versus the normal human control is associated with the presence of a gynecologic cancer such as ovarian, breast, uterine, endometrial or cervical cancer, or testicular cancer.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating
35 the patient being tested has cancer is one in which cells,

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tissues or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues or bodily fluid of a normal human control.

5 The present invention also provides a method of diagnosing metastatic gynecologic cancers or testicular cancer in a patient having such cancer which has not yet metastasized for the onset of metastasis. In the method of the present invention, a human cancer patient suspected of having a
10 gynecologic cancer or testicular cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a variety of means known to those of skill in the art.

In the present invention, determining the presence of CSG
15 levels in cells, tissues or bodily fluid, is particularly useful for discriminating between a gynecologic cancer or testicular cancer which has not metastasized and a gynecologic cancer or testicular cancer which has metastasized. Existing techniques have difficulty discriminating between gynecologic
20 cancers or testicular cancer which have metastasized and gynecologic cancers or testicular cancer which have not metastasized. However, proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker levels
25 measured in such cells, tissues or bodily fluid is CSG, and are compared with levels of CSG in preferably the same cells, tissue or bodily fluid type of a normal human control. That is, if the cancer marker being observed is CSG in serum, this level is preferably compared with the level of CSG in serum
30 of a normal human patient. An increase in the CSG in the patient versus the normal human control is associated with a gynecologic cancer or testicular cancer which has metastasized.

Without limiting the instant invention, typically, for
35 a quantitative diagnostic assay a positive result indicating

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the cancer in the patient being tested or monitored has metastasized is one in which cells, tissues or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues or bodily fluid of a normal patient.

Normal human control as used herein includes a human patient without cancer and/or non cancerous samples from the patient; in the methods for diagnosing or monitoring for metastasis, normal human control may also include samples from a human patient that is determined by reliable methods to have a gynecologic cancer or testicular cancer which has not metastasized such as samples from the same patient prior to metastasis.

15 **Staging**

The invention also provides a method of staging gynecologic cancers or testicular cancer in a human patient. The method comprises identifying a human patient having such cancer and analyzing a sample of cells, tissues or bodily fluid from such human patient for CSG. CSG levels in such cells, tissues or bodily fluid are then compared with levels of CSG in preferably the same cells, tissues or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the human patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Monitoring

Further provided is a method of monitoring gynecologic cancers or testicular cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues or bodily fluid from such human patient for CSG; comparing the CSG levels in such cells, tissues or bodily

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fluid with levels of CSG in preferably the same cells, tissues or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the human patient versus the normal human control is associated with a cancer which has
5 metastasized.

Further provided by this invention is a method of monitoring the change in stage of gynecologic cancers or testicular cancer in a human having such cancer. The method comprises identifying a human patient having such cancer;
10 periodically analyzing a sample of cells, tissues or bodily fluid from such human patient for CSG; comparing the CSG levels in such cells, tissues or bodily fluid with levels of CSG in preferably the same cells, tissues or bodily fluid type of a normal human control sample, wherein an increase in CSG
15 levels in the human patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease in the levels of CSG is associated with a cancer which is regressing in stage or in remission.

Monitoring such patient for onset of metastasis is
20 periodic and preferably done on a quarterly basis. However, this may be more or less frequent depending on the cancer, the particular patient, and the stage of the cancer.

Assay Techniques

Assay techniques that can be used to determine levels of
25 gene expression (including protein levels), such as CSG of the present invention, in a sample derived from a patient are well known to those of skill in the art. Such assay methods include, without limitation, radioimmunoassays, reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry
30 assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses, ELISA assays and proteomic approaches: two-dimensional gel electrophoresis (2D electrophoresis) and non-gel based approaches such as mass spectrometry or protein interaction profiling. Among these,

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ELISAs are frequently preferred to diagnose a gene's expressed protein in biological fluids.

An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to CSG, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to CSG. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, for example horseradish peroxidase enzyme or alkaline phosphatase.

To carry out the ELISA, antibody specific to CSG is incubated on a solid support, e.g. a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time CSG binds to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to CSG and linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to CSG. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to CSG antibodies, produces a colored reaction product. The amount of color developed in a given time period is proportional to the amount of CSG protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may be employed wherein antibodies specific to CSG attached to a solid support and labeled CSG and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of CSG in the sample.

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Nucleic acid methods may be used to detect CSG mRNA as a marker for ovarian and testicular cancer. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASABA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse-transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR can be used to identify the presence of a specific type of cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e. gridding) can be used to both detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the CSG gene is fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or plastic. At least a portion of the DNA encoding the CSG gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA, isolated from the tissue of interest. Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including but not limited to radioactive labeling or fluorescence labeling of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of gene expression can be done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the

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target gene, quantitating the yield, and then using that material to generate a standard curve.

Of the proteomic approaches, 2D electrophoresis is a technique well known to those in the art. Isolation of individual proteins from a sample such as serum is accomplished using sequential separation of proteins by different characteristics usually on polyacrylamide gels. First, proteins are separated by size using an electric current. The current acts uniformly on all proteins, so smaller proteins move farther on the gel than larger proteins. The second dimension applies a current perpendicular to the first and separates proteins not on the basis of size but on the specific electric charge carried by each protein. Since no two proteins with different sequences are identical on the basis of both size and charge, the result of a 2D separation is a square gel in which each protein occupies a unique spot. Analysis of the spots with chemical or antibody probes, or subsequent protein microsequencing can reveal the relative abundance of a given protein and the identity of the proteins in the sample.

The above tests can be carried out on samples derived from a variety of patients' cells, bodily fluids and/or tissue extracts (homogenates or solubilized tissue) such as from tissue biopsy and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum or any derivative of blood.

In Vivo Antibody Use

Antibodies against CSG can also be used *in vivo* in patients suspected of suffering from gynecologic cancers such as ovarian, breast, endometrial, uterine or cervical cancer or testicular cancer. Specifically, antibodies against a CSG can be injected into a patient suspected of having a gynecologic cancer or testicular cancer for diagnostic and/or therapeutic purposes. The use of antibodies for *in vivo*

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diagnosis is well known in the art. For example, antibody-chelators labeled with Indium-111 have been described for use in the radioimmunosintographic imaging of carcinoembryonic antigen expressing tumors (Sumerdon et al. Nucl. Med. Biol. 1990 17:247-254). In particular, these antibody-chelators have been used in detecting tumors in patients suspected of having recurrent colorectal cancer (Griffin et al. J. Clin. Onc. 1991 9:631-640). Antibodies with paramagnetic ions as labels for use in magnetic resonance imaging have also been described (Lauffer, R.B. Magnetic Resonance in Medicine 1991 22:339-342). Antibodies directed against CSGs can be used in a similar manner. Labeled antibodies against a CSG can be injected into patients suspected of having a gynecologic cancer or testicular cancer for the purpose of diagnosing or staging of the disease status of the patient. The label used will be selected in accordance with the imaging modality to be used. For example, radioactive labels such as Indium-111, Technetium-99m or Iodine-131 can be used for planar scans or single photon emission computed tomography (SPECT). Positron emitting labels such as Fluorine-19 can be used in positron emission tomography. Paramagnetic ions such as Gadolinium (III) or Manganese (II) can be used in magnetic resonance imaging (MRI). Localization of the label permits determination of the spread of the cancer. The amount of label within an organ or tissue also allows determination of the presence or absence of cancer in that organ or tissue.

For patients diagnosed with a gynecologic cancer or testicular, injection of an antibody against a CSG can also have a therapeutic benefit. The antibody may exert its therapeutic effect alone. Alternatively, the antibody is conjugated to a cytotoxic agent such as a drug, toxin or radionuclide to enhance its therapeutic effect. Drug monoclonal antibodies have been described in the art for example by Garnett and Baldwin, Cancer Research 1986 46:2407-2412. The use of toxins conjugated to monoclonal antibodies

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for the therapy of various cancers has also been described by Pastan et al. Cell 1986 47:641-648. Yttrium-90 labeled monoclonal antibodies have been described for maximization of dose delivered to the tumor while limiting toxicity to normal
5 tissues (Goodwin and Meares Cancer Supplement 1997 80:2675-2680). Other cytotoxic radionuclides including, but not limited to Copper-67, Iodine-131 and Rhenium-186 can also be used for labeling of antibodies against CSGs.

Antibodies which can be used in these in vivo methods
10 include both polyclonal, monoclonal or omniclonal antibodies and antibodies prepared via molecular biology techniques. Antibody fragments and aptamers and single-stranded oligonucleotides such as those derived from an in vitro evolution protocol referred to as SELEX and well known to
15 those skilled in the art can also be used.

EXAMPLES

The present invention is further described by the following examples. These examples are provided solely to illustrate the invention by reference to specific embodiments.
20 This exemplification, while illustrating certain aspects of the invention, does not portray the limitations or circumscribe the scope of the disclosed invention.

Example 1

Identification of the CSG of SEQ ID NO:1 was carried out
25 by a systematic analysis of data in the LIFESEQ database available from Incyte Pharmaceuticals, Palo Alto, CA, using the subsetting Search Tool of the LIFESEQ database available from Incyte Pharmaceuticals, Palo Alto, CA. Search Tools include: Library Comparison which compares one library to one
30 other library and allows the identification of clones expressed in tumor and absent or expressed at a lower level in normal tissue; Subsetting which is similar to library

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comparison but allows the identification of clones expressed in a pool of libraries and absent or expressed at a lower level in a second pool of libraries; and Transcript Imaging which lists all of the clones in a single library or a pool of libraries based on abundance. Individual clones are examined using Electronic Northernblots to determine the tissue sources of their component ESTs.

The following example was carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following example can be carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Example 2: Relative Quantitation of Gene Expression

Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control was used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) was used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" is obtained using

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the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

The tissue distribution and the level of the target gene in normal and cancer tissue were evaluated. Total RNA was extracted from normal tissues, cancer tissues and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was done using primers and Tagman probe specific to each target gene. The results are analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

Measurement of SEQ ID NO:1; Clone ID 1450626; Gene ID 236019 ("Pro104")

The absolute numbers depicted in Table 1 are relative levels of expression of Pro104 in 12 normal different tissues. All the values are compared to normal colon (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 1: Relative Levels of Pro104 Expression in Pooled Samples

Tissue	NORMAL
Colon Ascending	1.0
Endometrium	0
Kidney	0
Liver	0
Ovary	0
Pancreas	0
Prostate	1.0
Small Intestine	0
Spleen	0
Stomach	0
Testis	100
Uterus	0

The relative levels of expression in Table 1 show that Pro104 mRNA is not expressed in normal ovary. Its expression is,

- 19 -

however, higher (100) in testis compared with all the other normal tissues analyzed. Testis, with a relative expression level of 100, prostate (1.0), and colon (1.0) are the only tissues expressing mRNA for Prol04.

5 The absolute numbers in Table 1 were obtained analyzing pools of samples of a particular tissue from different individuals. They cannot be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 2.

10 The absolute numbers depicted in Table 2 are relative levels of expression of Prol04 in 70 pairs of matching and 30 unmatched tissue samples. All the values are compared to normal colon(calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from
15 the normal adjacent sample for that same tissue (NAT) from the same individual. In cancers (for example, ovary) where it was not possible to obtain normal adjacent samples from the same individual, samples from a different normal individual were analyzed.

20 **Table 2: Relative Levels of Prol04 Expression in Individual Samples**

Sample ID	Cancer Type	Tissue	Cancer	Matching or NAT
OVR10370/ 1038	Papillary serous adenocarcinoma	Ovary 1	187.3	0.6
25 OVR1305/ 13060	Papillary serous adenocarcinoma	Ovary 2	56.3	0
OVR9410C360	Endometrioid Adenocarcinoma	Ovary 3	0.9	
OVR14604A1C	Cancer	Ovary 4	9.1	
OVR11570	Papillary serous adenocarcinoma	Ovary 5	10.26	
30 OVR10400	Papillary serous adenocarcinoma	Ovary 6	169.3	
OVR14471A1B	Adenocarcinoma	Ovary 7	0.3	

- 20 -

	OVR10280	Ovarian carcinoma	Ovary 8	3.2	
	OVR11180	Small cell carcinoma	Ovary 9	0.05	
	OVR7730	Metastatic papillary serous adenocarcinoma	Ovary 10	1.3	
	OVR14603A1D	Adenocarcinoma	Ovary 11	3.5	
5	OVR10050	Papillary serous & endometrioid	Ovary 12	15	
	OVR9702C018 G	Normal cystic	Ovary 13		1.2
	OVR206I	Normal left atrophic, small cystic	Ovary 14		0.2
10	OVR9702C020 G	Normal, multiple ovarian cysts	Ovary 15		0.1
	OVR9702C025 G	Normal, hemorrhage CL cyst	Ovary 16		0.4
	OVR9701C035 G	Normal	Ovary 17		0
15	OVR9701C040 G	Normal, benign follicular cysts	Ovary 18		0
	OVR9701C050 G	Normal, multiple ovarian cysts	Ovary 19		0
20	OVR9701C109 R	Normal	Ovary 20		0
	OVR9702C004 G	Normal	Ovary 21		0
	OVR9702C007 R	Normal	Ovary 22		0
25	OVR9701C087 R	Normal, small follicle cysts	Ovary 23		0
	OVR9411C109	Normal	Ovary 24		0
	OVR9701C177 a	Normal, cystic	Ovary 25		0
30	OVR9701c179 A	Normal	Ovary 26		0.05
	OVR14610	Serous cystadenofibroma	Ovary 27		0
	OVR14638A1C	Right follicular cyst	Ovary 28		0
35	OVR9411C057 R	Benign large endometriotic cyst	Ovary 29		0

- 21 -

5	UTR13580/ 13590	Tumor/NAT	Uterus 1	0	0
	UTR14170/ 14180	Malignant tumor/NAT	Uterus 2	4.51	0.5
	UTR233U96/ 234U96	Adenocarcinoma/NAT	Uterus 3	4.24	1.67
	UTR850U/ 851U	Stage 1 endometrial cancer/NAT	Uterus 4	2.4	0
10	END10479B/ 10479D	Disease/NAT	Endometrium 1	15.2	0
	END9705A125 A/126	Endometrial carcinoma/NAT	Endometrium 2	4.67	0.53
	END9704C281 A/282	Endometrial adenocarcinoma/NAT	Endometrium 3	2.54	0.36
15	END14863A1A /A2A	Moderately differ. Endometr. carcin./NAT	Endometrium 4	0.32	0.31
	END9709C056 A/C055a	Adenocarcinoma of endometrium/NAT	Endometrium 5	0.41	0.29
	END	Invasive endometrial	Endometrium 6	0.09	1.36
20	END8911A/ 8911D	Disease/NAT	Endometrium 7	0	0.7
	END8963A/ 8963B	Disease/NAT	Endometrium 8	0	0
	END9807A080 A/081	Endometrial carcinoma/NAT	Endometrium 9	0.15	0.09
25	END9705A0/ 9705A0		Endometrium 10	0	0
	CVXVNM00/ VNM00		Cervix 1	0.1	0
	CVXVNM00/ VNM00		Cervix 2	0.4	0
30	CVXIND000/ IND000		Cervix 3	47.3	12.4
	CVXIND000/ IND000		Cervix 4	0.5	0.3
	MAMM826I/ 828I	Infiltrating ductal adenocarcinoma/NAT	Mammary 1	0.1	0
35	MAMM473P/ 475P	Disease/NAT	Mammary 2	0	0
	MAMM9706A06 6G/67	Disease/NAT	Mammary 3	0.08	0.17

- 22 -

5	MAMM9703B01 1d/b	Infiltrating ductal adenocarcinoma/NAT	Mammary 4	1.76	0.26
	MAMM0008603 M	Infiltrating ductal breast cancer	Mammary 5	0	
	MAMM9703A00 4B	Normal	Mammary 6		0 -
10	STM4004864/ 4864		Stomach 1	0.43	0.47
	STM4004509/ 4509		Stomach 2	0.7	51.94
	STM4004154/ 4154		Stomach 3	0.21	0.11
	STM4004317/ 4317		Stomach 4	0	0.12
15	CLN4004535A 2/B2	Asc. Colon, adenocarcinoma, stage D	Colon 1	0	0
	CLN	Rectum, Stage A	Colon 2	0	0
	CLN	Rectosigmoid, stage A	Colon 3	0	0
20	CLN9612B006 /B005	Asc. Colon, cecum, adenocarcinoma	Colon 4	0	0
	CLN703C091R /92RB	Rectosigmoid, stage T1	Colon 5	0	0
	LNG476Q/477 Q	Right Lung carcinoma	Lung 1	0	0
25	LNG605L/606 L	Right Lung carcinoma	Lung 2	0	0
	LNG750C/751 C	Metastatic osteogenic sarcoma	Lung 3	0	0
	PANC714L/ 715L	Villous adenoma	Pancreas 1	0	0
30	PANC824P/ 825P	Cystic adenoma	Pancreas 2	0	0
	PANC10343a/ 10343b		Pancreas 3	0.52	1.15
	PANC776P/ 777P		Pancreas 4	0.11	0.08
35	PANC9210/ 9220		Pancreas 5	109.81	0
	LIV150A/ 151A	Hepatocellular carcinoma	Liver 1	0	0
40					

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5	LIV942A/ 943A	Hepatoblastoma	Liver 2	0	0
	LIV12742B/ 12742C		Liver 3	0.06	0
	BLD327K/328 K	Papillary transitional cell carcinoma/NAT	Bladder 1	0	0
	BLD467K/468 K	Consistent w/ high grade urothelial carcin.	Bladder 2	0	0
10	BLD1496K/ 1497K		Bladder 3	0.6	0.2
	BLD1721K/ 1722K		Bladder 4	0	0
15	KID1064D/ 1065D	Tumor/NAT	Kidney 1	0	0
	KID1079D/ 1080D	Tumor/NAT	Kidney 2	0.3	0.4
	KID1097D/ 1098D	Tumor/NAT	Kidney 3	0	0
20	KID1263D/ 1264D	Tumor/NAT	Kidney 4	0	0
	KID512D/ 513D	Palpable renal cell carcinoma	Kidney 5	0.1	0.13
25	KID689D/690 D	Kidney carcinoma	Kidney 6	1.82	0
	KID988D/989 D	Renal cell carcinoma	Kidney 7	1.7	0.2
	KID1024D/ 1025D		Kidney 8	0	0
30	KID1183D/ 1184D		Kidney 9	0.7	0.1
	KID1229D/ 1230D		Kidney 10	0	9.2
35	PRO1291B/ 1292B	Adenocarcinoma/NAT	Prostate 1	11.9	0.2
	PRO209B/210 B	Adenocarcinoma of the prostate/NAT	Prostate 2	0	0
	PRO1222B/ 1223B	Adenocarcinoma/NAT	Prostate 3	19.9	3.2
40	PRO1293B/ 1294B	Adenocarcinoma/NAT	Prostate 4	0.7	4.4
	PRO650B/651 B	Adenocarcinoma/NAT	Prostate 5	1.34	0.15

- 24 -

	PRO694B/695 B		Prostate 6	0	0
	PRO780B/781 B	Adenocarcinoma/NAT	Prostate 7	0.2	0.8
5	PRO845B/846 B	Adenocarcinoma/NAT	Prostate 8	0.2	0
	PRO902B/903 B		Prostate 9	0	0
10	PRO916B/917 B	Tumor/NAT	Prostate 10	0	0
	PRO1012B/ 1013B		Prostate 11	3.27	2.74
	PRO139B/140 B		Prostate 12	0.19	0.01
15	TST239X/240 X	Tumor/NAT	Testis 1	1.66	8.19

0= Negative

In the analysis of all samples, the higher levels of expression were in tumor ovary (median 6.3), showing a high degree of specificity for ovarian cancer. Expression of Prol04 was also found in other cancers of the female reproductive tissues including the cervix, endometrium, uterus, and breast. The median level in these tissues was 3.2). Of all the samples (other than the female reproductive tissues) analyzed, only one sample (the cancer sample Pancreas 5 with 109.81) showed an expression comparable to the mRNA expression in ovarian cancer. The median for the expression of Prol04 for all other cancer tissues was zero.

The level of mRNA expression in cancer samples and from the isogenic normal adjacent tissue from the same individual or normal adjacent tissue from a different individual were also compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 2 shows an induction of Prol04 expression in 2 ovary cancer tissues compared with their respective normal adjacent (ovary samples # 1, 2) and, the induction of

- 25 -

Pro104 in 10 unmatched cancer tissues (ovary samples #3 through 12) compared with 17 normal adjacent tissues (ovary samples 13 through 29). There was induction of Pro104 expression in the cancer tissue for 100% of the ovary samples tested (total of 2 matching and 10 unmatched ovary samples). However, 71% of the normal adjacent tissue samples were negative, and 29% (5 of 17) showed a low level of Pro104 expression of 0.05 - 1.2 normalized units.

Altogether, the absence of expression in 71% of the 10 normal adjacent tissues (with negligible expression in the remaining 29% normal adjacents), plus the higher level of mRNA expression in 100% of the ovary tumor samples tested is indicative of Pro104 being a diagnostic marker for ovarian cancer. Expression of this marker in the cervix, endometrium, 15 uterus, and mammary cancers, is indicative of P104 also being a diagnostic marker in other gynecologic cancers.

Based on the amino acid composition as depicted in SEQ ID NO:2, Pro104 is a serine protease that shares 37% homology with human hepsin at the nucleotide level, and 31% homology 20 at the level of amino acids. When aligned with several other serine proteases such as kallikrein-1, kallikrein-2, prostate specific antigen, prostasin, protease M, and hepsin, Pro104 shares all the conserved amino acid motifs that are characteristic of all other serine proteases. For example, 25 Pro104 contains a highly conserved RIVGG (SEQ ID NO:3) sequence. Cleavage between arginine (R) and isoleucine (I) results in the activation of proteases that contain the motif (Kurachi et al. Hepsin. Methods in Enzymology, Vol. 244, pp. 100-114 (1994)). The active protease domain of Pro104 30 therefore consists of 273 amino acids. Pro104 also contains the conserved histidine, aspartic acid, and serine residues that form a catalytic triad for enzymatic function.

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What is claimed is:

1. A method for diagnosing the presence of a gynecologic cancer or testicular cancer in a patient comprising:

5 (a) measuring levels of CSG in cells, tissues or bodily fluids in a patient; and

(b) comparing the measured levels of CSG with levels of CSG in cells, tissues or bodily fluids from a normal human control, wherein a change in measured levels of CSG in said
10 patient versus normal human control is associated with the presence of a gynecologic cancer or testicular cancer.

2. A method of diagnosing metastases of a gynecologic cancer or testicular cancer in a patient comprising:

(a) identifying a patient having a selected cancer that
15 is not known to have metastasized;

(b) measuring CSG levels in a sample of cells, tissues, or bodily fluid from said patient; and

(c) comparing the measured CSG levels with levels of CSG in cells, tissue, or bodily fluid of a normal human control,
20 wherein an increase in measured CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

3. A method of staging a gynecologic cancer or testicular cancer in a patient having a gynecologic cancer or
25 testicular cancer comprising:

(a) identifying a patient having the cancer;

(b) measuring CSG levels in a sample of cells, tissue, or bodily fluid from said patient; and

(c) comparing measured CSG levels with levels of CSG in
30 cells, tissues, or bodily fluid of a normal human control sample, wherein an increase in measured CSG levels in said patient versus the normal human control is associated with a cancer which is progressing and a decrease in the measured CSG

- 27 -

levels is associated with a cancer which is regressing or in remission.

4. A method of monitoring a gynecologic cancer or testicular cancer in a patient for the onset of metastasis comprising:

- (a) identifying a patient having a gynecologic cancer or testicular cancer that is not known to have metastasized;
- (b) periodically measuring levels of CSG in samples of cells, tissues, or bodily fluid from said patient for CSG; and
- 10 (c) comparing the periodically measured CSG levels with levels of CSG in cells, tissues, or bodily fluid of a normal human control, wherein an increase in any one of the periodically measured CSG levels in the patient versus the normal human control is associated with a cancer which has
- 15 metastasized.

5. A method of monitoring the change in stage of a gynecologic cancer or testicular cancer in a patient comprising:

- (a) identifying a patient having a gynecologic cancer or
- 20 testicular cancer;
- (b) periodically measuring levels of CSG in cells, tissues, or bodily fluid from said patient for CSG; and
- (c) comparing the periodically measured CSG levels with
- 25 human control, wherein an increase in any one of the periodically measured CSG levels in the patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease is associated with a cancer which is regressing in stage or in remission.

30 6. The method of claim 1, 2, 3, 4 or 5 wherein the CSG comprises SEQ ID NO:1.

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7. An antibody against a CSG wherein said CSG comprises
SEQ ID NO:1.

8. A method of imaging a gynecologic cancer or
testicular cancer in a patient comprising administering to the
5 patient an antibody of claim 7.

9. The method of claim 8 wherein said antibody is
labeled with paramagnetic ions or a radioisotope.

10. A method of treating a gynecologic cancer or
testicular cancer in a patient comprising administering to the
10 patient an antibody of claim 7.

11. The method of claim 10 wherein the antibody is
conjugated to a cytotoxic agent.

SEQUENCE LISTING

<110> Shujath, Ali M.
Cafferkey, Robert
DIADEXUS LLC

<120> Method of Diagnosing, Monitoring, Staging, Imaging and
Treating Gynecologic Cancers and Testicular Cancer

<130> DEX-0044

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5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21774

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 39/395; G01N 33/48, 33/574; C07K 16/00; C12Q 1/68

US CL : 424/138.1, 139.1, 181.1; 435/6, 7.23; 436/64; 530/387.7

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/138.1, 139.1, 181.1; 436/64; 530/387.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN. EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/36054 (AMRAD OPERATIONS PTY. LTD.) 20 August 1998, abstract	1-11
A	INUOE, M, et al Cloning and distribution of a novel serine protease esp-1 from human eosinophils, Biochemical and Biophysical Research Communications, 1998, Vol 252, pages 307-312, especially page 307.	1-11
Y	Database Genbank, Accesion No. AC005361, RICKE,D., Homo sapiens chromosome 16, cosmid clone 352F10 (LANL), complete sequence, 01 August 1998, see complete sequence.	1-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

16 DECEMBER 1999

Date of mailing of the international search report

10 FEB 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

JENNIFER E. NICHOLS, NEE HUNT

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21774

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/21774

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-9, drawn to an antibody against a CSG, wherein CSG comprises SEQ ID NO:1, and a method of use — in diagnosing, staging, monitoring, and imaging cancer using CSG.

Group II, claim(s) 10-11, drawn to a second method of use for treating cancer using CSG.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

An international stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories:

1. a product and process specially adapted for the manufacture of said product; or
2. a product and process of use of said product; or
3. a product, a process specially adapted for the manufacture of said product and a use of said product; or
4. a process and an apparatus or means specifically designed for carrying out the process; or
5. a product, process specially adapted for the manufacture of said product, and an apparatus or means specifically designed for carrying out the process.

The inventions of Groups I and II are drawn to completely different methods of use which are not connected by a special technical feature. The special technical feature appears to be CSG comprising SEQ ID NO:1. The claims of Group I are drawn to a method of using CSG, and a product, an anti-CSG antibody. The claims of Group II are drawn to a completely different method of using CSG. The methods have completely different objectives, different starting materials, different method steps, and different outcomes.